

## Polyclonal Antibody to RFP-Tag (Ads. to Hu, Ms, Rt Serum Proteins) - FITC

<b>Alternate names:</b>	DsRed Tag, Red fluorescent protein Tag
<b>Catalog No.:</b>	AP09229FC-N
<b>Quantity:</b>	0.1 mg
<b>Concentration:</b>	1.0 mg/ml (by UV absorbance at 280 nm)
<b>Background:</b>	Epitope tagging is a powerful and versatile strategy for detecting and purifying proteins expressed by cloned genes. To utilize this feature, protein expression vectors are typically engineered with a nucleotide sequence that encodes the peptide epitope tag. The gene of interest is cloned in-frame relative to the tag and, upon expression, the protein of interest is synthesized as a fusion protein with the peptide tag. Fusion protein detection and/or purification is mediated by highly specific antibodies to the engineered peptide, thus eliminating the need for antibodies to proteins from each newly cloned gene. Commonly used epitope tags include glutathione-S-transferase (GST), c-myc, 6-histidine (6X-His), FLAG, green fluorescent protein (GFP), red fluorescent protein (RFP, DsRed), maltose binding protein (MBP), influenza A virus haemagglutinin (HA), b-galactosidase, and GAL4.
<b>Uniprot ID:</b>	<a href="#">Q9U6Y8</a>
<b>NCBI:</b>	<a href="#">86600</a>
<b>Host / Isotype:</b>	Rabbit / IgG
<b>Immunogen:</b>	Red Fluorescent Protein (RFP) fusion protein corresponding to the full length amino acid sequence (234aa) derived from the mushroom polyp coral <i>Discosoma</i>
<b>Format:</b>	<b>State:</b> Lyophilized purified Ig fraction <b>Purification:</b> Immunoaffinity Chromatography using Red Fluorescent Protein ( <i>Discosoma</i> ) coupled to agarose beads followed by solid phase adsorption(s) to remove any unwanted reactivities <b>Buffer System:</b> 0.02M Potassium Phosphate, 0.15M Sodium Chloride, pH 7.2 containing 10 mg/ml BSA (Immunoglobulin and Protease free) as stabilizer and 0.01% (w/v) Sodium Azide as preservative <b>Label:</b> FITC – Fluorescein isothiocyanate (Molecular Weight 390 daltons) <i>Absorption / Emission:</i> 495 nm / 528 nm <i>Molar Ratio:</i> 2.2 moles FITC per mole of Rabbit IgG <b>Reconstitution:</b> Restore with 0.1 ml of deionized water (or equivalent).
<b>Applications:</b>	Antibodies to RFP ( <i>Discosoma</i> spp.) are intended for use in immunological assays including ELISA, Western blotting, Fluorometry and Fluorescence activated cell sorting (FACS). Polyclonal anti-RFP is designed to detect RFP and its variants. This antibody can be used to detect RFP by ELISA (sandwich or capture) for the direct binding of antigen. Biotin

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conjugated polyclonal anti-RFP used in a sandwich ELISA with unconjugated anti-RFP is well suited to titrate RFP in solution. The detection antibody conjugated to biotin is subsequently reacted with streptavidin conjugated HRP. Fluorochrome conjugated polyclonal anti-RFP can be used to detect RFP by immunofluorescence microscopy in cell expression systems and can detect RFP containing inserts. Significant amplification of signal is achieved using fluorochrome conjugated polyclonal anti-RFP relative to the fluorescence of RFP alone. For immunoblotting use either alkaline phosphatase or peroxidase conjugated polyclonal anti-RFP to detect RFP or RFP containing proteins on western blots.

Recommended Dilutions:

FLISA: > 1/20,000.

Western blot: > 1/10,000.

Immunofluorescence: 1/500-1/2,5000.

Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

**Specificity:**

Expect reactivity against RFP and its variants: mCherry, tdTomato, mBanana, mOrange, mPlum, mOrange and mStrawberry. Assay by Immunoelectrophoresis resulted in a single precipitin arc against anti-Rabbit Serum and purified and partially purified Red Fluorescent Protein (Discosoma).

No reaction was observed against Human, Mouse or Rat serum proteins. ELISA was used to confirm specificity at less than 0.1% of target signal.

**Storage:**

Prior to reconstitution store at 2-8°C.

Following reconstitution store undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer.

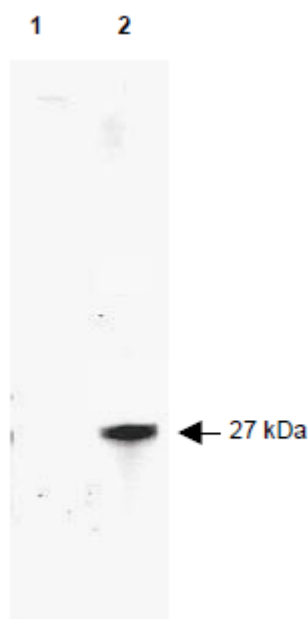
Avoid repeated freezing and thawing.

Shelf life: one year from despatch.

**General References:**

1. The and Feltkamp, Immunology 18; 865, 1970 (Conjugation).
2. Gross LA, Baird GS, Hoffman RC, Baldrige KK, Tsien RY: The structure of the chromophore within DsRed, a red fluorescent protein from coral. Proc. Natl. Acad. Sci. USA 2000, 97: 11990-11995.

**Pictures:**



Western blot of RFP recombinant protein detected with anti-RFP antibody. Lane 1 shows no reaction against a GFP recombinant protein present in 10 µg of HeLa cell extract. Lane 2 shows a single band detected in 10 µg of a HeLa lysate containing RFP recombinant protein. Anti-RFP detects a 27 kDa band corresponding to the epitope tag RFP. A 4-12% Bis-Tris gradient gel (Invitrogen) was used for SDS-PAGE. The protein was transferred to nitrocellulose using standard methods. After blocking the membrane was probed with the primary antibody diluted 1:2,500 for 1 h at room temperature followed by washes and reaction with a 1:5,000 dilution of IRDye(TM)800 conjugated Goat-a-Rabbit IgG [H&L]. IRDye(TM)800 fluorescence image was captured using the Odyssey® Infrared Imaging System developed by LI-COR. IRDye is a trademark of LI-COR, Inc. Other detection systems will yield similar results.

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